

AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph on page 2, lines 20-23 as follows:

It is contemplated that new CBD's can be found by cloning cellulases, xylanases or other plant cell wall degrading enzymes and measure the binding to e.g. cellulose. If the enzyme activity is bound to Avicel® under the standard conditions described below, it can be assumed that part of the gene codes for a binding domain.

Please amend the paragraphs on page 3, lines 11-15 as follows:

Apart from the fungal CBM's of family CBM1 which have binding affinity for cellulose, the CBM of the invention is the first known fungal CBM shown to have binding affinity for cellulose.

The inventors have succeeded in cloning and expressing a CBM bound to a family GH61 enzyme. In addition, the inventors have expressed the domain only, without the GH61 enzyme and demonstrated that the CBM alone can bind cellulose, such as Avicel®.

Please amend the paragraph on page 7, lines 24-33 as follows:

The expressed CBM or CBM-containing polypeptide of the invention has a molecular weight (Mw) which is equal to or higher than about 15 kD in an unglycosylated form. The majority of the protein binding to Avicel® appeared as a broad band of molecular weight 35-45 kDa, which is considerably higher than the 15 kDa of the protein part of the carbohydrate binding module. The high and heterogeneous molecular weight is probably due to heterogeneity in O- and N-glycosylation of the N-terminal part of the protein. For heterologously expressed CBMX in *Aspergillus oryzae* the size of CBMX can vary from 14 kDa to almost 70 kDa due to heterologous glycosylation of the protein. Moreover, N-terminal sequencing of the 35-45 kDa band gave exclusively the sequence SFSSSGT (positions 47-53 of SEQ ID NO:9) indicating that heterogeneity in the N-terminal amino acid sequence is not present.

Please amend the paragraph on page 29, lines 1-3 as follows:

The produced CBM may be detected using methods known in the art and modifications thereof that are specific for the CBM. These detection methods may include use of specific antibodies or determination of binding to a carbohydrate substrate, such as Avicel®.

Please amend the paragraph on page 35, line 30 – page 36, line 6 as follows:

The *Aspergillus oryzae* strain described in Example 1B expressing the CBM (CBMX) of the *Pseudoplectania nigrella* GH61 with *Candida antarctica* lipase signal peptide was grown in shake flasks. About 1 liter culture broth was sterile filtered and the filtrate loaded onto a column containing 50 g Avicel®. Non-binding and weakly binding proteins were removed by washing with Milli-Q® water. Proteins with affinity for Avicel® were eluted with 0.1 M Tris, pH 11.5. Immediately after elution, pH of this Avicel®-binding fraction was adjusted to 7.5, and the fraction was concentrated using an Amicon® cell (Millipore®, USA) with a membrane having a cut-off of 6 kDa. On SDS-PAGE the majority of the protein binding to Avicel® appeared as a broad band of molecular weight 35-45 kDa, which is considerably higher than the molecular weight of the protein part of the carbohydrate binding module. The high and heterogeneous molecular weight is probably due to heterogeneity in O- and N-glycosylation of the N-terminal part of the protein. N-terminal sequencing of the 35-45 kDa band gave exclusively the sequence SFSSSGT (positions 47-53 of SEQ ID NO:9) indicating that heterogeneity in the N-terminal amino acid sequence is not present.

Please amend the paragraphs and table on page 35, line 30 – page 37, line 5 as follows:

The carbohydrate-binding domain with affinity for Avicel® and purified as described in Example 2 (CBMX) was studied further. 50 µl purified CBMX was mixed with 500 µl 20 mM Tris, pH 7.5 containing varying amount of Avicel® (0-100 mg/ml) in an Eppendorf tube. After 4 hours incubation at room temperature with agitation, the samples were centrifuged. 200 µl supernatant was transferred to the well of a microtiter plate (Costar, UV plate) and absorbance read at 280 nm on a microtiter plate reader (SpectraMax® Plus, Molecular Devices). The results in Table 1 indicate that the large majority of the protein binds to the highest concentrations of Avicel®.

Table 1: Binding of CBMX to Avicel®. A280: Absorbance at 280 nm of 200 µl supernatant in microtiter plate with absorbance of buffer and Avicel subtracted.

Avicel® (mg/ml)	A280
100	0.0095
50	0.0185
25	0.0326
12.5	0.0540
6.25	0.0637
3.125	0.0729
1.563	0.0799
0.781	0.0808
0.391	0.0777
0.0	0.0768

Experiments with shorter incubation time (15 min to 1 hour) gave less complete binding.

A similar binding study was performed with PASC (Phosphoric Acid Swollen Cellulose: To 5 g Avicel® moistened with water 150 ml ice-cold 85 ortho-phosphoric acid is added. After 1 hour stirring on ice bath, 500 ml cold acetone is added. The suspension is filtered and washed, first with acetone and then with water). 50 µl purified CBMX was mixed with 500 µl 20 mM Tris, pH 7.5 containing varying amount of PASC (0-10 mg/ml) in an Eppendorf tube. Samples were incubated 4 hours at room temperature with agitation. After centrifugation, 200 µl supernatant was transferred to the well of a microtiter plate and absorbance read at 280 nm. The results in Table 2 show that increasing amount of PASC reduces the amount of absorbance of CBMX in the supernatant, i.e. CBMX has affinity for PASC.

Please amend the paragraph on page 37, lines 10-17 as follows:

Affinity of CBMX for a number of soluble carbohydrates was tested in a competition assay by mixing 100 µl CBMX with both Avicel® (400 µl 50 mg/ml in 20 mM Tris, pH 7.5) and the soluble carbohydrate (dissolved in 500 µl 20 mM Tris, pH 7.5). As references, samples without CBMX or soluble carbohydrate added were used. If CBMX has affinity for the soluble carbohydrate it should be able to keep CBMX in solution which can be measured as increase in absorbance at 280 nm compared to sample without soluble carbohydrate added. After 4 hours

incubation at room temperature with agitation, samples were centrifuged and absorbance at 280 nm was read using 200 µl supernatant in the well of a microtiter UV plate.

Please amend the paragraph and table on page 37, line 29 – page 38, line 10 as follows:

Table 3: Competition binding assay with CBMX, Avicel® (20 mg/ml) and soluble carbohydrates. Concentration of carbohydrate: Concentration of soluble carbohydrate during incubation with CBMX and Avicel®. Difference in A280: Difference in absorbance at 280 nm between samples with and without CBMX added.

Soluble carbohydrate	Carbohydrate conc. (mg/ml)	Difference in A280
None - Only Avicel® and CBMX		0.018
None - Only CBMX		0.108
beta-Glucan	20	0.100
Lichenan	20	0.025
CMC	25	0.129
Xyloglucan	11	0.052
Galactan	20	0.043
Locust bean gum	25	0.087